A Selective Synthesis of 4-Aminobiphenyl-N^2-deoxyguanosine Adducts

Susanne Scheer, Thomas Steinbrecher, and Gernot Boche

Fachbereich Chemie der Universitat, Marburg, Germany

A selective synthesis of N^2-deoxyguanosine adducts derived from 4-aminobiphenyl (ABP) is described. The reactions of O^2-trifluoromethylsulfonyl-O^6-allyl-3',5'-*O*-bis(*tert*-butyldimethylsilyl)-2'-deoxyxanthosine with 3-amino-4-acetaminobiphenyl and 4-hydrazinobiphenyl, respectively, are the key steps. Successive removal of the protecting groups from the protected adducts leads to the free adducts 3-(deoxyguanosine-N^2-yl)-acetyl-ABP and *N*-(deoxyguanosyl-N^2-yl)-ABP, respectively. — Environ Health Perspect 102(Suppl 6):151–152 (1994)

Key words: carcinogenic aromatic amines, 4-aminobiphenyl-N^2-deoxyguanosine adducts; C-2 activated purine deoxyribonucleosides

Introduction

Electrophilic metabolites of aromatic amines like 4-aminobiphenyl (ABP) form preferentially deoxyguanosine adducts upon reaction with DNA. The major adducts are *N*-(deoxyguanosine-C-8-yl)-ABP (C-8-dG-ABP) 1 and the *N*-acetylated C-8-dG-AABP 2. Minor adducts are 3-(deoxyguanosine-N^2-yl)-acetyl-ABP (N^2-dG-3-AABP) 3 and *N*-(deoxyguanosine-N^2-yl)-ABP (N^2-dG-*N*-ABP) 4 (*1,2*) (Figure 1).

In connection with our program aimed at the synthesis of adducts of purine bases, we were interested in a selective synthesis of N^2-deoxyguanosine (dG) adducts of arylamines. Strategically, two routes can be envisaged (Figure 1): amination of dG with electrophiles such as *O*-2,6-dichlorobenzoyl-*N*-acetyl-*N*-(4-biphenyl)hydroxylamine (*3*), or *O*-acetyl-*N*-(4-biphenyl)-hydroxylamine (*4*) (bond formation A); this protocol, however, leads mostly to adduct formation at C-8 of the purine (*3,4*) and a substitution reaction of an electrophilic, C-2 activated nucleoside with nucleophilic arylamines (arylhydrazines) R'-NH_2 (bond formation B). Recently Lee et al. (*5*) reported on the reaction of the electrophilic O^2-2-fluoro-O^6-(*p*-nitrophenylethyl)-2'-deoxyinosine with an arylmethylamine. Based on our experience with the synthesis of alkylated N^2-deoxyguanosine derivatives, we selected the fully protected O^2-trifluoromethanesulfonyl-O^6-allyl-3',5'-bis(*tert*-butyldimethylsilyl)-2'-deoxyxanthosine 5 (*6*), which has the better leaving group at the C-2 position of the purine moiety (Table 1).

Reaction of the activated deoxyxanthosine 5 with 3-amino-4-acetaminobiphenyl 6 led to a 30% yield of the corresponding protected N^2-deoxyguanosine adduct 10. We confirmed this type of reaction with 1,2-diaminobenzol 7 to give 11 (Table 1). Due to the lack of the acetyl group in 7, its reaction with 5 was much faster than that of 5 with 6; also 11 was formed in higher yields than 10. The other structural type of N^2-deoxyguanosine adduct was accessible by reacting 5 with the hydrazine 8 leading to the protected adduct 12 in 77% yield. In the same manner the fluorene-derived hydrazine 9 reacted with 5 to give the protected adduct 13 in 69% yield.

To demonstrate the facile removal of the protecting groups in adducts like 10 to 13, the protected adduct 10 was first reacted with tetrabutylammoniumfluoride (TBAF) to remove the *tert*-butyldimethylsilyl groups, and then with Wilkinson's catalyst $(Ph_3P)_3RhCl$ to remove the allyl group to give the free ABP adduct 3 in 50% yield. Similarly, the protected adduct 12 was transformed into the free adduct 4 in 40% yield.

In summary, the preceding protocol allows the selective synthesis of N^2-dG adducts derived from aromatic amines that otherwise are only accessible in very low yields and together with C-8 adducts.

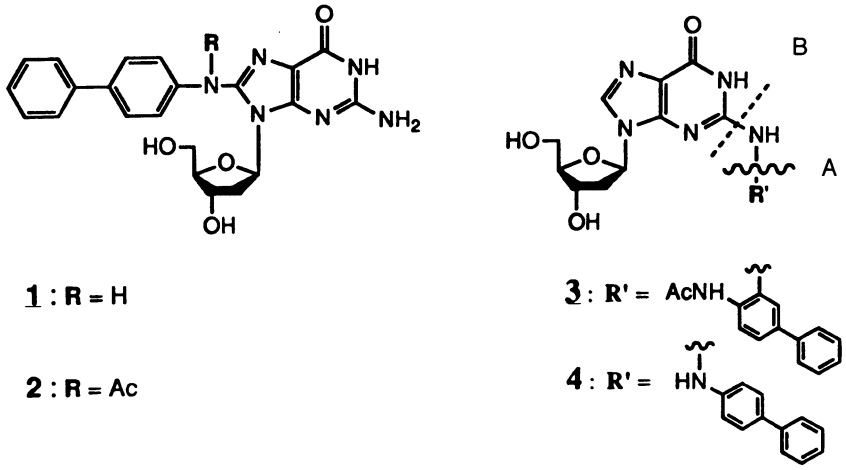


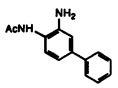
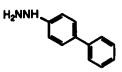
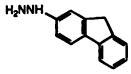
Figure 1. N^2-Deoxyguanosine adducts derived from 4-aminobiphenyl.

This paper was presented at the Fifth International Conference on Carcinogenic and Mutagenic *N*-Substituted Aryl Compounds held 18–21 October 1992 in Würzburg, Germany.

T.Steinbrecher is grateful to the Deutsche Forschungs gemeinschaft for a postdoctoral grant.

Address correspondence to Gernot Boche, Fachbereich Chemie der Universitat, Hans-Meerwein-Straβe, D-35032 Marburg, Germany. Telephone 06421 282030. Fax 06421 285547.

Table 1. Reaction conditions and yields for the reactions of the activated deoxyxanthosine 5 with the arylamines 6 and 7, and the arylhydrazines 8 and 9, respectively.

Arylamine or arylhydrazine *RNH*[2]	Reaction time and temperature	Yields of the fully protected adducts
6 	3 days, 80°C	30% of 10
7 	3 days, 20°C	70% of 11
8 	14 days, 20°C	77% of 12
9 	14 hr, 20°C	69% of 13

Materials and Methods

Chemicals

3-Amino-4-acetaminobiphenyl 6 is accessible by catalytic hydrogenation of 3-nitro-4-acetaminobiphenyl (*7*) with Pd/C in ethanol in quantitative yield. 4-Hydrazinobiphenyl 7 was synthesized according to Müller (*8*).

Synthesis of the Protected Arylamine and Arylhydrazine N^2-deoxyguanosine Adducts 10 to 13

100 mg (0.13 mmole) of the hypoxanthine derivative 5 was dissolved in 5 ml DMF and stirred with a 10-fold excess of arylamine 6 (*7*) or arylhydrazine 8 (*9*). The reaction was monitored by TLC. After completion, the solvent was evaporated under vacuum and the crude product was purified by flash chromatography using a mixture of petrol ether and ethyl acetate (3:1, vol/vol).

Desilylation of 10 and 12

The disilyl compounds (0.1 mmole) were stirred at room temperature for 1.5 hr with TBAF (1.1 M solution in THF, 4 equiv). Then the mixture was evaporated to dryness. The resulting crude products were purified by flash chromatography using a mixture of $CHCl_3$ and MeOH (9:1, vol/vol).

Deallylation of the Desilylated 10 and 12

The allyl-protected adducts (0.1 mmole) were refluxed in 10 ml ethanol to which a few drops of water were added in the presence of a catalytic amount of $(Ph_3P)_3RhCl$. The reactions were monitored by TLC. After completion the solvent was evaporated under vacuum. The crude products were purified by recrystallization from water/ethanol (1:1, vol/vol) (*3*), and by chromatography (silica gel; $CHCl_3/CH_3OH$, 8:2, vol/vol) (*4*). Yields: 10 → 3:50%; 12 → 4:40%.

3-(Deoxyguanosine-N^2-yl)-4-acetylaminobiphenyl 3. ^{1}H-NMR (400 MHz, d_6-DMSO, D_2O) δ [ppm] 8.51 (s, 1H, H_2) 8.08 (s, 1H, purine) 7.68 (d, 2H, $H_{2'}$,$H_{6'}$) 7.50 (pseudo-t, 2H, $H_{3'}$, $H_{5'}$) 7.37 (pseudo-s, 2H, $H_{5''}$, $H_{6'}$) 7.35 (t, 1H, $H_{4'}$) 6.26 (pseudo-t, 1H, $H_{1''}$) 4.39 (m, 1H, $H_{3''}$) 3.85 (m, 1H, $H_{4''}$) 3.46–3.40 (m, 2H, $H_{5''}$, $H_{5''\star}$) 2.60–2.57 (m, 1H, $H_{2''}$) 2.26–2.22 (m, 1H, $H_{2''\star}$) 2.11 (s, 3H, OAc).

N-*(Deoxyguanosine-N^2-yl)-4-aminobiphenyl 4.* ^{1}H-NMR (400 MHz, d_6-DMSO, D_2O) δ [ppm] 7.97 (s, 1H, NH) 7.95 (s, 1H, purine) 7.59 (d, 2H, $H_{2'}$, $H_{6'}$) 7.52 (d, 2H, H_2, H_6) 7.41 (pseudo-t, 2H, $H_{3'}$, $H_{5'}$) 7.28 (t, 1H, $H_{4'}$) 6.92 (d, 2H, H_3, H_5) 6.17 (pseudo-t, 1H, $H_{1''}$) 4.37 (m, 1H, $H_{3''}$) 3.85 (m, 1H, $H_{4''}$) 3.55 (m, 2H, $H_{5''}$,$_{5''\star}$) 2.54 (m, 1H, $H_{2''}$) 2.23 (m, 1H, $H_{2''\star}$).

REFERENCES

1. Beland FA, Kadlubar FF. Formation and persistence of arylamine DNA adducts *in vivo*. Environ Health Perspect 62:19–30 (1985).
2. Gupta RC, Dighe NR. Formation and removal of DNA adducts in rat liver treated with *N*-hydroxy derivatives of 2-acetylaminofluorene, 4-acetylaminobiphenyl, and 2-acetaminophenanthrene. Carcinogenesis 5:343–349 (1984).
3. Underwood GR, Price MF, Shapiro R. A new synthetic route to nucleotide adducts derived from *N*-acetylated and unacetylated 4-aminobiphenyl. Carcinogenesis 9:1817–1821 (1988).
4. Famulok M, Bosold F, Boche G. Synthese von *O*-acetyl-*N*-(4-biphenylyl)hydroxylamin, einem entscheidenden metaboliten des carcinogenen 4-aminobiphenyls, und seine reaktion mit desoxyguanosin. Angew Chem 101:349–350 (1989).
5. Lee H, Hinz M, Stezowski JJ, Harvey RG. Synthesis of polycyclic aromatic hydrocarbon nucleoside and oligonucleotide adducts specifically alkylated on the amino functions of deoxyguanosine and deoxyadenosine. Tetrahedron Lett 31:6773–6776 (1990).
6. Steinbrecher T, Wameling C, Oesch F, Seidel A. Die Trifluormethansulfonatfunktion als neue Aktivierung der C2-Position am Purin: Synthese von N^2-alkylierten Desoxyguanosinen. Angew Chem 105:404–405 (1993).
7. Fichter F, Sulzberger A. Über das Phenylbenzochinon und einige Derivate des Biphenyls. Chem Ber 37:879–884 (1904).
8. Muller H. Über p-Hydrazinodiphenyl. Chem Ber 27:3105–3108 (1894).
9. Corey EJ, Suggs J.W. Selective cleavage of allylethers under mild conditions by transition metal reagents. Org Chem 38:3224 (1973).